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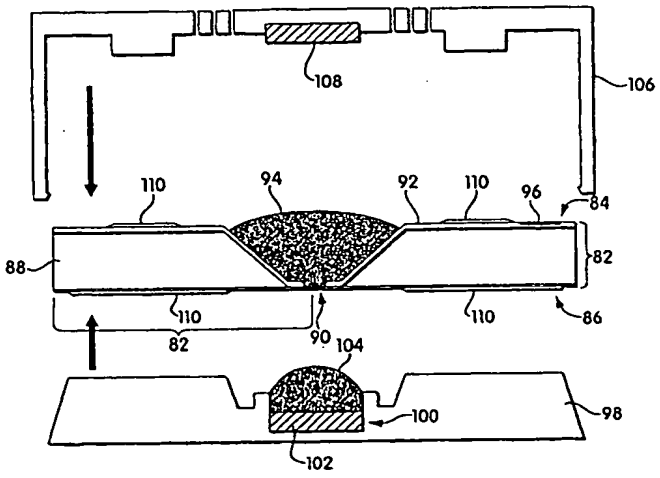
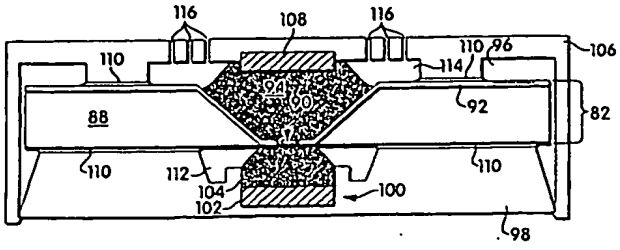
(54) Title: BIOLOGICAL ION CHANNELS IN NANOFABRICATED DETECTORS

(57) Abstract

The present invention relates to a device for generating an oscillating electrical current, where the device incorporates an ion channel. In particular, the ion channel is incorporated into an integrated electronic device having nanoscale dimensions. Thus, this device can transform biological processes into an electrical output. The present invention also describes a sensor for detecting biological or chemical analytes with the ion channel device. Methods for generating the oscillating currents and detecting the analytes are also disclosed.





BIOLOGICAL ION CHANNELS IN NANOFABRICATED DETECTORS

Field of the Invention

The present invention relates to a biological/electronic interface, and more particularly to a device for generating an oscillating electrical current that incorporates a biological ion channel.

Background of the Invention

Nature has devised a large number of methods to transport or conduct charge across biological interfaces. Accordingly, there has been a concerted effort to exploit this biological conductivity by either (1) preparing synthetic mimics of the biological conductor or (2) by using the actual biological conductor. The second approach is particularly attractive because many of these biological species have structures that are too sophisticated to easily mimic. Such *in vitro* use, however, can present the disadvantage of a lack of stability of the biological species when placed in an unnatural environment.

One example of transporting charge in biology occurs when ions are conducted across cell membranes through membrane proteins, for example ion channels or ion pumps. With an ion channel, the ions move through the channel in a thermodynamically downhill direction. In the case of ion pumps, the ions travel through the pumps in a thermodynamically uphill direction, and thus need an energy source to carry out this energetically unfavorable process.

Fig. 1 illustrates a schematic example of a membrane 2. The membrane comprises a lipid bilayer 4 having, interspersed within, biological species 6 having a pore 7, of an ion channel, allowing transport of ions from one side of the membrane to the other side. For example, ions can move from an area outside of a cell membrane, to an area inside of the membrane.

The movement of ions through the channels or pumps is not a free-flowing motion of ions, but rather the membrane regulates the flow of ions. Fig. 2 shows a similar diagram as Fig. 1, but illustrating the distribution of charges inside and outside of a membrane wall. Typically, the inside of a membrane is negatively charged, i.e., the inside has an excess of negatively charged species, whereas the outside of a membrane is positively charged. The inside of a membrane can have a potential of between about -60 mV to about -100 mV relative to the outside. Due to this separation of charged species, the membrane is said to be in a "polarized state". When the membrane is polarized to a threshold extent, the pore 7 of

One such device known in the art is a patch clamp. Fig. 3A illustrates a typical patch clamp. The patch clamp 10 comprises a glass pipet 11 having an electrolyte solution 13. The inset of Fig. 3A shows an expanded view of the tip of the pipet. The tip features a lipid membrane 15 which extends across the diameter of the tip. Membrane 15 includes an ion channel pore 16. The membrane can be a single cell or comprise protein reconstituted within a lipid bilayer. Typically, the diameter of the tip is 1 μm. As shown in the inset, the glass pipet has one electrolyte solution 13 situated on one side of membrane 15 and electrolyte solution 14 situated on the other side of membrane 15. Electrodes 19 and 20 can be immersed into electrolyte solution 13 and 14 respectively, where the electrodes are also connected to amplifier head 18.

Fig. 3B shows a plot of oscillating electrical current as a function of time. As time progresses, short bursts of electrical current are generated. These bursts can range in the order of milliseconds to seconds, depending on the oscillating frequency. The patch clamp represented a significant advancement in the field, especially by providing increased sensitivity.

The principles of the patch clamp have been used to prepare several other related devices. U.S. Patent No. 5,516,890 (Tomich et al.) and U.S. Statutory Invention Registration No. H201 (Yager) both relate to patch clamp-type devices. Yager teaches incorporating proteins into synthetic membranes and Tomich discloses the use of synthetic proteins that mimic ion channels. U.S. Patent Nos. 5,503,744 and 5,378,342 (both Ikematsu et al.) relate to biological oscillating devices comprising a lipid membrane having ion pumps, where the membrane is situated between two electrolyte solutions. The device is activated by an energy source such as light. U.S. Patent No. 5,225,374 (Fare et al.) relates to a sensor. The sensor includes a porous semiconductor substrate having a lipid bilayer with receptor or protein pores, where the bilayer is positioned on the substrate.

While the above and other reports represent, in many cases, useful biological/electronic interfaces, there remains a need to prepare devices for generating oscillating electrical currents having increased sensitivity and lifetimes. In addition, there exists a need to fabricate such devices in nanoscale dimensions. In addition, sensors for detecting various biological or chemical analyzers need to be developed to detect analytes at very low concentrations with increased sensitivity.

Another device of the invention includes a barrier having a first side and a second side. A pore is located in the barrier, which can exist in an open state or a closed state. The closed state prevents ionic communication across the pore and the open state allows ionic communication across the pore from the first side of the barrier to the second side. An electrolyte container, constructed and arranged to contain an electrolyte and to position the electrolyte in contact with the first side of the pore is provided, and a second electrolyte container, constructed and arranged to contain an electrolyte and to position the electrolyte in contact with a second side of the pore, is fastenable to the first electrolyte container.

In another embodiment a device of the invention includes a barrier having two sides and including a pore, a described in the above paragraph. A first electrolyte container, constructed and arranged to contain an electrolyte and to position the electrolyte in contact with the first side of the pore is fastenable to the barrier. A second electrolyte container, also fastenable to the barrier, is constructed and arranged to contain an electrolyte and to position the electrolyte in contact with a second side of the pore.

In another embodiment a device includes a barrier having a first side and a second side, and a pore in the barrier as described in the above paragraph. A first electrolyte container includes container interior walls integral with the barrier, and a second electrolyte container also contains container interior walls integral with the barrier.

In another aspect a series of methods is provided. One method involves providing one or more membranes each positioned between two electrolyte reservoirs. Each membrane has at least one oscillating ion channel. The method involves measuring an electrical output from at least one oscillating ion channel in each membrane, or simultaneously measuring an electrical output from two or more oscillating ion channels.

Another method of the invention involves detecting a sample of analyte. The method involves providing at least one ion channel oscillating at a first frequency. A sample is allowed to bind to the at least one ion channel to cause the channel to oscillate at a second frequency, and the second frequency then is measured.

Another method of the invention involves sensing an analyte. In the method, an ion channel is allowed to oscillate at a relatively steady frequency for a period of time of at least about one second. Then, the ion channel is exposed to an analyte that affects the oscillation frequency of the channel and this change is detected indicating presence of the analyte.

Fig. 4A illustrates a schematic representation of an ion channel within a lipid membrane, where the ion channel is formed from a circular array of protein subunits;

Fig. 4B shows a helical representation of bovine F_0 subunit c, as modeled from a possible structure of E. Coli;

Fig. 5 shows a proposed mechanism for oscillation of a sodium/calcium ion channel;

Fig. 6A shows a side view of a schematic representation of a biological oscillating device;

Fig. 6B shows a top view of the device of Fig. 6A;

Figs. 7A and 7B show schematic representations of a sensor disposed on a chip, where the sensor has an array of 16 holes having membranes containing ion channels;

Figs 8A and 8B show photocopies of scanning electron micrograph (SEM) images of nanofabricated holes in SiN_x membranes, patterned by direct-write electron beam lithography and reactive ion etching;

Fig. 9 shows plots of current vs. time where the ion pore is located within the nanofabricated device;

Fig. 10 illustrates schematically a sensor according to one embodiment of the invention;

Figs. 11A and 11B illustrate schematically a sensor according to yet another embodiment of the invention; and

Fig. 12 illustrates, schematically, a hole within a barrier, including a barrier thin film, lipid bilayer membrane, and biological ion channel of a device according to one embodiment of the invention.

Detailed Description

The present invention relates to electronic/biological interface devices having improved sensitivity, accuracy, and/or packaging. The devices can convert biological charge transport processes at ion channels into an electrical output. The invention includes sensor packaging arrangements that are simple, compact, easy to manufacture in bulk, and facilitate exposure of both sides of ion channels to different electrolyte solutions. The invention also provides devices having one or more holes in an insulator, each including an ion channel, to provide statistical accuracy and increased signal intensity; small holes, allowing increased sensitivity and an ability to fabricate nanoscale devices; and amplification of output electrical signal.

one electrolyte reservoir and one end of the negative bias electrode partially immersed in the other electrolyte reservoir. The other ends of the electrodes can be connected to a plurality of electrical instruments, such as a voltage source for applying a voltage and a current detector for measuring current. Application of a voltage can cause a change in the membrane potential, allowing the "open state" to occur and transport of charge through the pore to provide electrical current.

The device of the invention can be constructed as a sensor with the electrical circuitry set to conditions that provide a detectable current. In one embodiment, applying a voltage of between about 60 mV to about 100 mV generates a current of at least about 10 pA, preferably at least about 50 pA, more preferably at least about 100 pA and even more preferably at least about 200 pA. The device can include an amplifier to amplify the magnitude of the generated current. This embodiment provides an additional method to maximize the amount of current.

In preferred embodiments, devices of the invention include a single pore, in a membrane positioned within a small hole of an insulating barrier. In such an arrangement, small holes are desired. Accordingly, a device having nanoscale dimensions, such as the dimensions found in a silicon chip, with a pore-containing hole having a diameter of less than about 1 μm, preferably less than about 500 nm, and more preferably less than about 200 nm, is preferred.

Accuracy of the device can be improved by obtaining a statistical number of electrical events. Toward that end, one embodiment provides an insulating layer having at least two holes and membranes comprising at least one pore positioned within each of the holes. Each of the holes can have a diameter as described previously. The at least two holes can be an array of holes, such as an n x m matrix where n and m can be the same or different and at least one of n and m is an integer of at least 2. Where a single pore exists in each hole, this arrangement provides an n x m matrix array of holes, and of pores. Arrays of essentially any size can be used, including arrays of 8 x 8 or larger. When the arrays comprise a large number of holes, providing holes of small diameters as described above can be especially advantageous. Such a device can simultaneously generate an oscillating current from at least two pores and, consequently, simultaneously measure the current from the at least two pores. Where two or more pores are arranged in a single device (i.e., a single pore within each of two or more holes in an insulating barrier), a common electrolyte can be positioned on one

for 1 day, the ion channel can be incorporated into the device and generate an oscillating electrical current. In another embodiment, the ion channel has sufficient stability allowing it to be effective in an operative device for at least one day, that is, being electrically connected so as to oscillate constantly for at least one day.

Fig. 5 shows a proposed mechanism for oscillation in a sodium/calcium ion channel. In (a), the negative potential side of the membrane has a low calcium concentration (less than 200 nm) which provides the pore in an open state. In this configuration, the pore can conduct mainly sodium current together with a small amount of calcium current. This conduction results in a build-up of calcium ion concentration on the negative potential side of the membrane (b). In (c) the high calcium concentration on the negative potential side of the membrane causes the pore to close. This closure results from the cooperative binding of several calcium ions to the pore, thought to be at least four calcium ions. After calcium diffusion from the ion channel, (d) shows the reconfiguration of the pore in an open state where the negative potential side once again has a low calcium ion concentration, as in Fig. 5(a).

Thus, the particular ion channels discussed in Fig. 5 have the advantageous feature of cooperative regulation by a number of calcium ions, or at least four calcium ions. The cooperative feature is significant, especially when considering that chemical energy is generated by the binding of each calcium ion on each protein subunit. For example, the binding of six calcium ions, where the binding of each calcium ion results in an energy gain of 0.5 eV, can produce a net energy total of 3 eV. As shown in the inset graph, this cooperative binding also results in a sharp transition between the open and closed state. A sharp transition allows the oscillation to occur very rapidly, which can provide increased resolution with respect to time.

Figs. 6A and 6B show schematic side and top views, respectively, of one embodiment of a device in accordance with the present invention. The device can be a sensor, a device for generating an oscillating current, or the like. The device is fabricated as a chip, as would be understood to those of ordinary skill in the art. In Fig. 6A, device 50 has an electrically insulating barrier defined by a silicon substrate 51 carrying a thin film insulating layer 52 (e.g. silicon nitride) positioned in electrical communication with an electrical circuit that is constructed and arranged to determine a change in an electrical characteristic across insulating layer 52. Specifically, the insulating layer is positioned between two electrolytes 54 and 55. Insulating layer 52 includes a hole 53 passing between the two electrolyte

separate reservoirs. Thus, each membrane can provide ionic communication between the same two electrolyte reservoirs, through at least one oscillating ion channel, or provide ionic communication between individual electrolyte reservoirs to a common reservoir. In one embodiment, the method involves an array of holes. The method provides a simultaneous measurement of electrical output caused by the oscillating ion channels which provide an oscillating flow of charge. In one embodiment, the method can involve providing a device as previously described.

Another advantage of this method lies in the fact that the application of a voltage results in the oscillating electrical current. Thus, by applying a constant voltage the ion channel can oscillate. In one embodiment, the ion channel oscillates steadily for at least one day, i.e. the ion channel may cease to oscillate momentarily but the ion channel is capable of restarting the oscillations.

As mentioned, one aspect of the invention provides a sensor for detecting a sample of an analyte. The sensor includes an ion channel having the attributes described previously. In one embodiment, the ion channel is ligand-gated. By "ligand-gated," any biological or chemical species that is capable of interacting or binding to the ion channel causes a change in the oscillation frequency, and examples of such biological or chemical species are disclosed in "Biochemistry" by L. Stryer (W.H. Freeman and Co., NY, 1995) which is hereby incorporated by reference in its entirety. Each analyte will change an ion channel's oscillating frequency to a second frequency that can be higher or lower than the initial or first frequency. Thus, the sensor operates under the principle that a particular analyte is detected when the second oscillation frequency occurs.

In one embodiment, the sensor includes a device for generating an oscillating current, as described previously, where the device includes at least one ion channel positioned within a barrier separating two electrolytes. An analyte can bind to an ion channel, changing its frequency of oscillation, and allowing sensing. For example, one electrolyte reservoir is exposed to an atmosphere suspected of containing the analyte. When the analyte eventually reaches the electrolyte, it diffuses through the electrolyte and eventually binds to the ion channel. The oscillating frequency of the ion channel can then change to a second frequency that can depend on the manner and extent of binding or interaction between the ion channel and the analyte.

In one embodiment, the sensor includes a detection instrument for detecting the change in frequency. In another embodiment, when the sensor is constructed for a particular

in oscillation is indicative of a detectable change, such as presence of an analyte. Certain prior art devices, in contrast, require an activation step to "turn on" the device (begin oscillations), where the activation step can involve exposure to an energy source, such as light. Because the present invention does not require a separate activation step to turn on the sensor, analytes can be detected "passively" as opposed to "actively." When an analyte is "actively" sensed, the operator is controlling the sensor and monitoring the sensor for the presence of the device. When an analyte is "passively" sensed, the sensor does not require monitoring. Passive sensors can be applicable when there is a need to detect, for example, a noxious biological or chemical species that is suspected to be present within the general area. Thus, a passive sensor does not require constant monitoring, but upon detection of a particular biological or chemical analyte, the sensor can generate a signal that indicates the presence of the analyte. Thus, one aspect of the invention is a method that involves long-term operation of an ion channel in an oscillating state, for example, at least one hour, at least one day, or at least one week, and after this period of time exposing the sensor to an analyte and allowing the oscillation frequency of the sensor to change and to be detected.

Figs. 7A and 7B schematically illustrate a sensor in accordance with the present invention having an array of holes, each of which can contain an ion channel pore, fabricated using standard silicon technology with microholes made lithographically. Fig. 7(a) shows a side view of one hole in chip 70. Chip 70 includes an SiN_x insulating barrier 71 having hole 72. In hole 72 resides a membrane having at least one pore. On the other side of the hole is a second electrolyte solution 73 which can comprise an extremely small volume such as a volume from a pipet tip. A silicon layer 74 can be positioned on the insulating layer 71 except in the area around hole 72. The silicon layer 74 can then be overlaid with a second insulating layer 75 (SiO_2). Electrode 76 can then positioned on insulating layer 75 such that electrode 76 is in contact with electrolyte solution 73.

Fig. 7(b) schematically illustrates a top view of a sensor chip 70 having an array of holes 72. The array of holes can be positioned on one side in a common electrolyte bath, and on the other side in contact with separate electrolyte baths 77 as shown in Fig. 7(b). Of particular interest in Fig. 7(b) is the presence of a series of amplifiers 78, for example gain stages, connected to each of holes 72. These amplifiers allow amplification of an oscillating electric current generated from the device. Thus, one aspect of the invention is an amplifier electrically connected to an ion channel. The array shown in Fig. 7(b) is not an n x m array, but rather an array outlining a square, to simplify showing connection of each hole to the

constructed to snap-fit together, sandwiching therebetween the middle portion of the device including barrier 82. Seals, such as Sylgard® seals 110 can be provided to mate with portions of bottom component 98 and top component 106 of device 80 to create isolated chambers containing electrolytes immediately above and below hole 90. When device 80 is assembled, electrolyte 94 and electrolyte 104 are brought into contact with opposite sides of hole 90 in barrier 82, thus in contact with opposite sides of the ion channel (not shown) within hole 90. Electrical circuitry (not shown) connects electrodes 102 and 108 for obtaining measurements as described above. Device 80, when assembled, includes a sealed bottom chamber 112 that contains electrolyte 104 and is bordered by electrode 102, interior surfaces of bottom component 98, the bottom side of silicon nitride film 110, and the bottom side of the lipid bilayer membrane and ion channel within hole 90. As illustrated, electrolyte 104 does not completely fill chamber 112. Instead, chamber 112 also includes air outside of electrolyte 104 that allows for expansion and contraction of electrolyte 104 upon variation in temperature. A top chamber 114 is defined upon assembly of the device that includes electrolyte 94 and is bordered by the top side of barrier 82, an interior surface of top component 106, and the top side of silicon nitride film and the lipid bilayer and pore within hole 90. Chamber 114 also is not completely filled by electrolyte 94, but includes air outside of the boundary of electrolyte 94. When assembled, electrolyte 104 is in contact with electrode 102, and electrolyte 94 is in contact with electrode 108, each electrolyte being in contact with the pore within hole 90. Top component 106 includes passages 116 within a wall thereof for exposure of electrolyte 94 to a fluid suspected of containing an analyte that can interact with the pore within hole 90 to affect oscillation frequency. When the sensor is exposed to air containing such an analyte, for example, the analyte passes through passages 116, diffuses through electrolyte 94, binds to the pore within hole 90, and its presence is sensed.

Figs. 11A and 11B illustrate, schematically, another sensor device 120 of the invention. Device 120 is similar to devices 50 and 80 of Figs. 6A-6B and 10, respectively. Fig. 11B is a top view of sensor 122, and Fig. 11A is a cross-section through lines B-B of Fig. 11B, showing a barrier 122 separating electrolytes 124 and 126 within bottom and top containers 128 and 130, respectively, defined by connection of bottom component 132 and 134, respectively, to barrier 122. As illustrated, bottom component 132 defines, itself, an electrode addressed by an electrical lead 136, and top component 134 defines an electrode addressed by an electrical lead 138. Electrolyte solution 124 completely fills bottom

by annular soft plastic member 150, annular thin film 154 within void 152 of member 150, and annular lipid bilayer membrane 158 within hole 156 of thin film 154.

The function and advantage of these and other embodiments of the present invention will be more fully understood from the examples below. The following examples are intended to illustrate the benefits of the present invention, but do not exemplify the full scope of the invention.

Example

This example describes the preparation of a device incorporating a biological oscillating ion channel. The ion channel comprised an array of the subunit c of ATP synthase. Isolation of this ion channel was performed as reported in Brain Research, Vol. 766, pp. 188-894 (1997, McGeoch et al.).

The ion channel was positioned within a hole of a 250 nm thick SiN_x insulating layer. The dimensions of the hole were 130 nm x 180 nm, the hole being patterned by direct-write electron beam lithography and reactive ion etching. Figs.8 A and B shows photocopies of SEM images of nanofabricated holes in SiNx membranes. Fig 8A shows 130 x 180nm hole in a 250nm thick SiNx membrane which was patterned by direct write electron beam lithography and reactive ion etching. Fig. 8B shows a 31nm hole in a 1.1μm thick SiN_x membrane which was patterned by focused ion beam milling.

Fig. 8 shows a photocopy of an SEM of this nanofabricated hole.

This insulating layer was incorporated into a device as shown in Fig. 6A. The bilayers of reconstituted protein in lipid vesicles and electrolytes were prepared as described in McGeoch et al (p. 189, section 2.4). The silicon layer had dimensions of 12 mm x 12 mm x 1 mm and the silicon nitride layer had dimensions of 4 mm x 4 mm x 250 nm. The electrolyte solutions were contained in a 4 mm x 4 mm x 4 mm teflon holder.

Fig. 9 shows a current vs. time plot, indicating the oscillation of the ion channel in the device. The oscillation frequency can be varied as shown in plots (a) and (b). Fig. 9 shows that the same oscillating current is obtained in the SiN_x barrier holes of the invention as is present in prior art patch clamp assays involving a glass micropipette barrier with a one micron hole. In plot (a), the SiN_x barrier was 250 nanometers thick and the hole was of dimension 130 x 180 nanometers in diameter. Plot (b): SiN_x barrier 1.1 micron thick and a hole of 50 nanometers diameter. Both holes were patterned by focus ion beam milling.

Those skilled in the art would readily appreciate that all parameters listed herein are meant to be exemplary and that actual parameters will depend upon the specific application

CLAIMS

1. A device for generating an oscillating current, comprising:

an insulating layer positioned between at least two electrolyte reservoirs;

a negative bias electrode and a positive bias electrode, each electrode having one end in electrical communication with respective electrolyte reservoirs, the other ends of the electrodes being connected to a voltage source for applying a voltage and a current detector for measuring current;

at least one hole penetrating the insulating layer;

at least one pore positioned within each of the at least one hole, the at least one pore existing in one of an open and a closed state, wherein the closed state prevents ionic communication between the reservoirs and the open state allows ionic communication between the reservoirs to generate electrical current.

2. A device as in claim 1, wherein the at least one hole has a diameter of less than about 1 μm.

3. A device as in claim 1, wherein the at least one hole has a diameter of less than about 500 nm.

4. A device as in claim 1, wherein the at least one hole has a diameter of less than about 300 nm.

5. A device as in claim 1, wherein the at least one hole has a diameter of less than about 100 nm.

6. A device as in claim 1, wherein the at least one pore has a diameter of less than about 10 angstroms.

7. A device as in claim 1, wherein the at least one pore has a diameter of between about 3 angstroms and about 10 angstroms.

8. A device as in claim 1, further comprising the at least one pore being positioned in a lipid bilayer positioned within each of the at least one hole.

applying a voltage of between about 60 mV to about 100 mV.

21. A device as in claim 1, wherein the current has a value of at least about 200 pA upon applying a voltage of between about 60 mV to about 100 mV.

22. A device as in claim 1, further comprising an array of holes penetrating the insulating layer, and a separate electrolyte reservoir contacting each hole on at least one side of the insulating layer.

23. A device as in claim 22, wherein the array of holes is an n x m array and n and m can be the same or different and each of n and m is an integer of at least 2.

24. A device as in claim 1, further comprising an amplifier to amplify the generated electrical current.

25. A device for generating an oscillating current, comprising an oscillating ion channel, wherein the ion channel is positioned within a membrane spanning a hole having a diameter less than 1 μm.

26. A device as in claim 1, wherein the at least one hole has one common electrolyte reservoir.

27. A method, comprising:

providing at least one membrane positioned between two electrolyte reservoirs, the membrane having at least one oscillating ion channel, and measuring an electrical output from the oscillating ion channel in the membrane.

28. A method as in claim 27, wherein the ion channel oscillates steadily for at least 1 day.

29. A method as in claim 27, wherein the ion channel is selected from the group consisting of a sodium ion channel, a potassium ion channel, a calcium ion channel and combinations thereof.

immersing one end of each of a negative bias electrode and a positive bias electrode into respective electrolyte reservoirs, the other ends of the electrodes being connected to a voltage source for applying a voltage and a detector for measuring current.

38. A method as in claim 36, wherein a time between the binding and measuring the second frequency is less than about 1 s.

39. A method as in claim 36, wherein a time between the binding and measuring the second frequency is less than about 500 ms.

40. A method as in claim 36, wherein a time between the binding and measuring the second frequency is less than about 100 ms.

41. A method as in claim 36, wherein the amount of analyte in the sample is less than about 1 nM.

42. A method as in claim 36, wherein the amount of analyte in the sample is less than about 500 pM.

43. A method as in claim 36, wherein the amount of analyte in the sample is less than about 100 pM.

44. A method as in claim 36, further comprising derivatizing the ion channel with functional groups to detect a predetermined analyte.

45. A method as in claim 36, wherein the first frequency is at least 0.1 Hz.

46. A device comprising:
an ion channel capable of oscillation; and
an electrical amplifier in electrical communication with the ion channel.

47. A device as in claim 46, further comprising an electrical insulator, wherein the ion channel is located in a hole in the barrier passing from a first side of the insulator to a second

a pore in the barrier, existing in one of an open and a closed state, the closed state preventing ionic communication across the pore and the open state allowing ionic communication across the pore from the first side of the barrier to the second side of the barrier;

a first electrolyte container, fastenable to the barrier, constructed and arranged to contain an electrolyte and to position the electrolyte in contact with a first side of the pore; and

a second electrolyte container, fastenable to the barrier, constructed and arranged to contain an electrolyte and to position the electrolyte in contact with a second side of the pore.

51. A device as in any of claims 48-50, wherein the barrier includes an electrical insulator.

52. A method for generating at least one oscillating current, comprising providing at least two separate membranes positioned adjacent at least one electrolyte reservoir, each membrane having at least one oscillating ion channel, and simultaneously measuring an electrical output from the at least one oscillating ion channel in each membrane.

53. A device comprising:

a first electrolyte reservoir;

a second electrolyte reservoir;

electrical circuitry connecting the first and second electrolyte reservoirs; and

subunit c of ATP synthase separating first and second electrolyte reservoirs.

54. A device or method as in any preceding claim, including a hole spanned by an insulating membrane containing a pore.

55. A device or method as in any preceding claim, including subunit c of ATP synthase or a derivative.

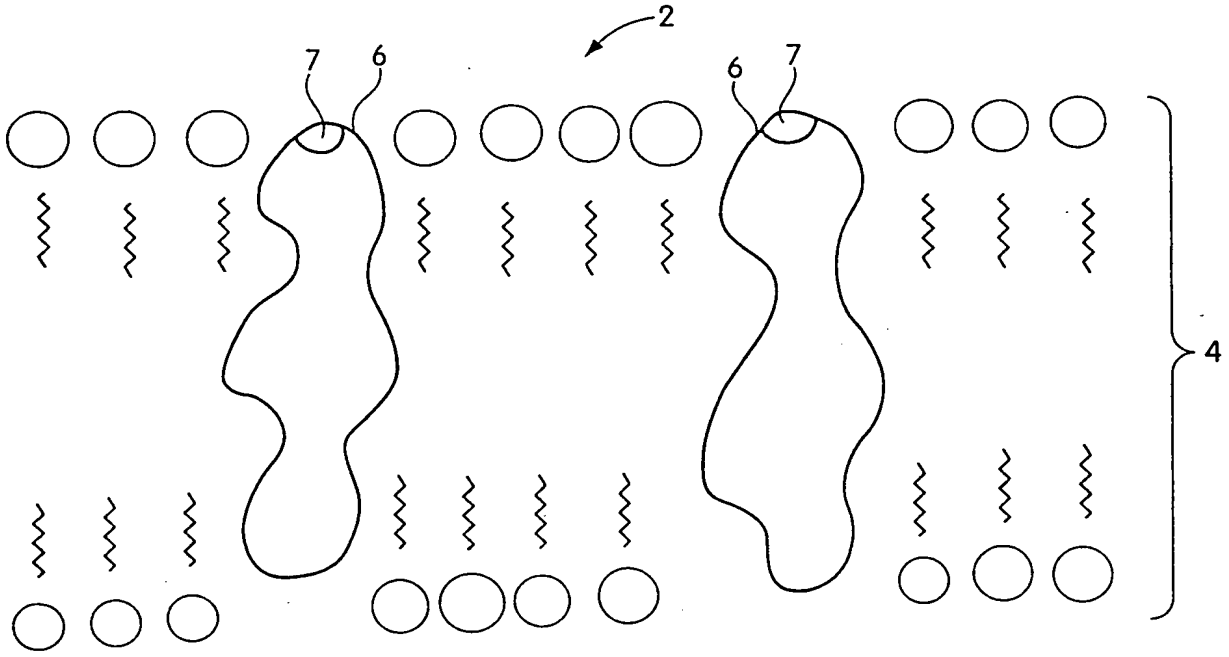


Fig. 1
(PRIOR ART)

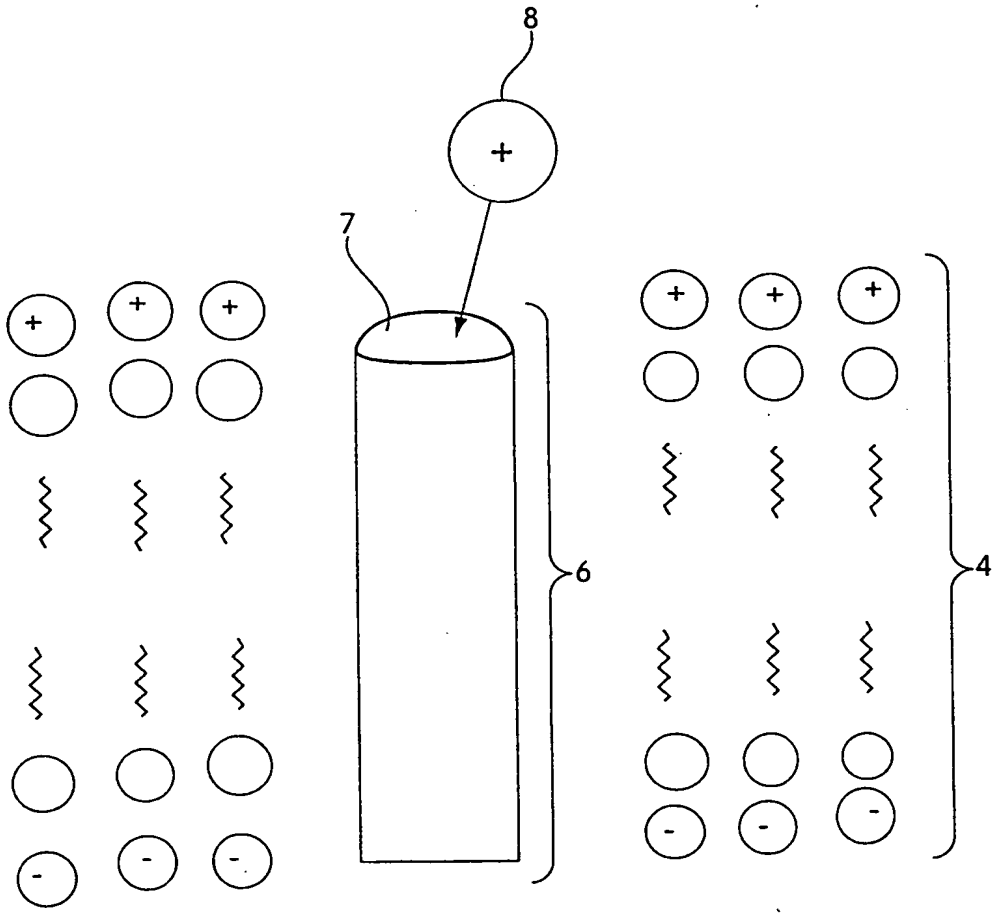


Fig. 2
(PRIOR ART)

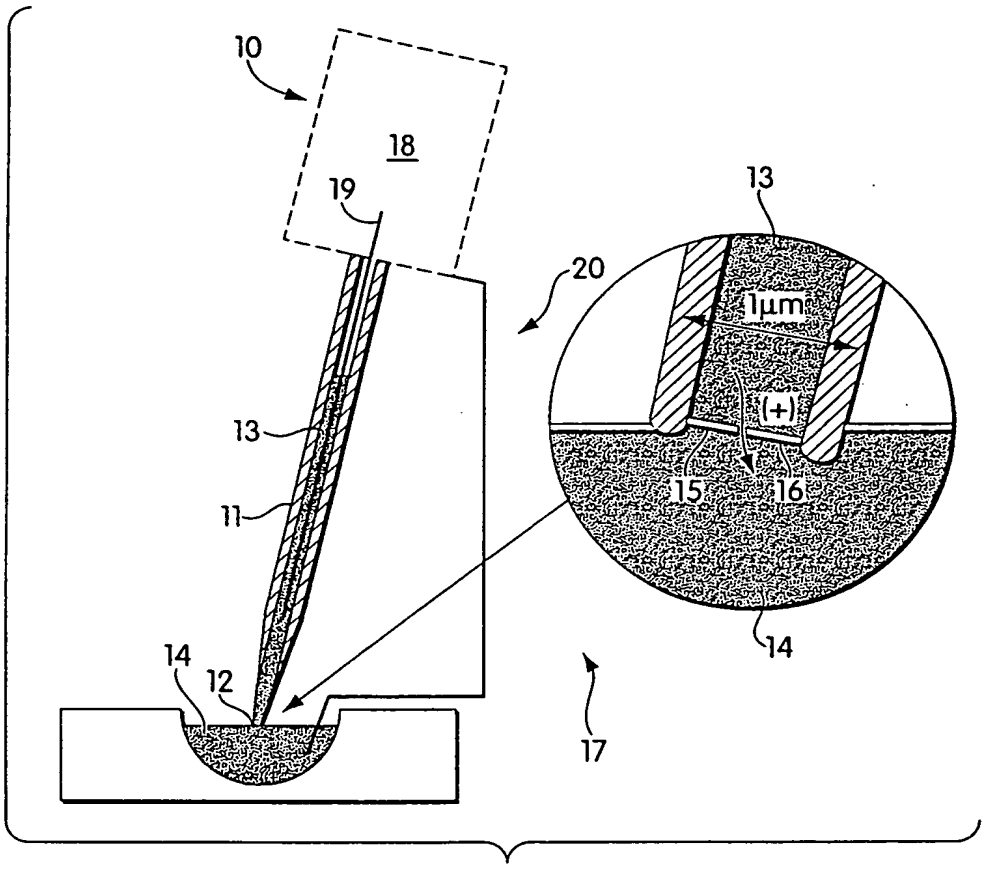


Fig. 3A
(PRIOR ART)

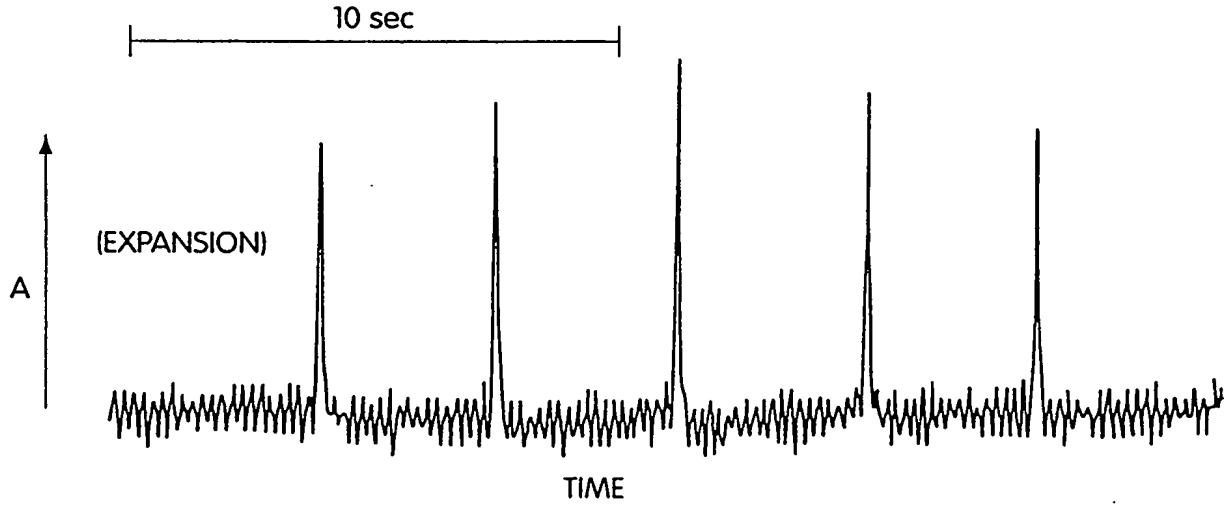


Fig. 3B
(PRIOR ART)

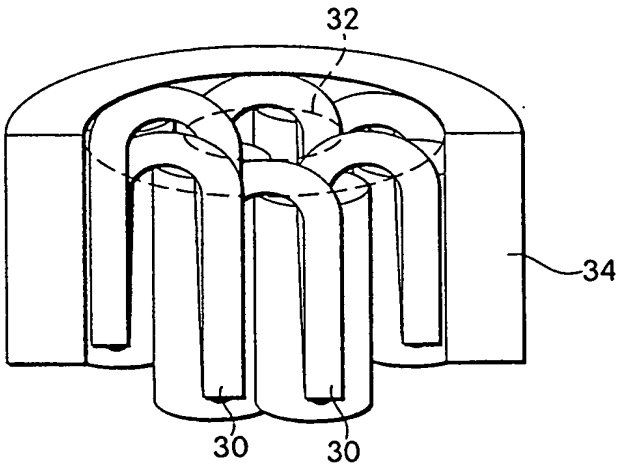


Fig. 4A

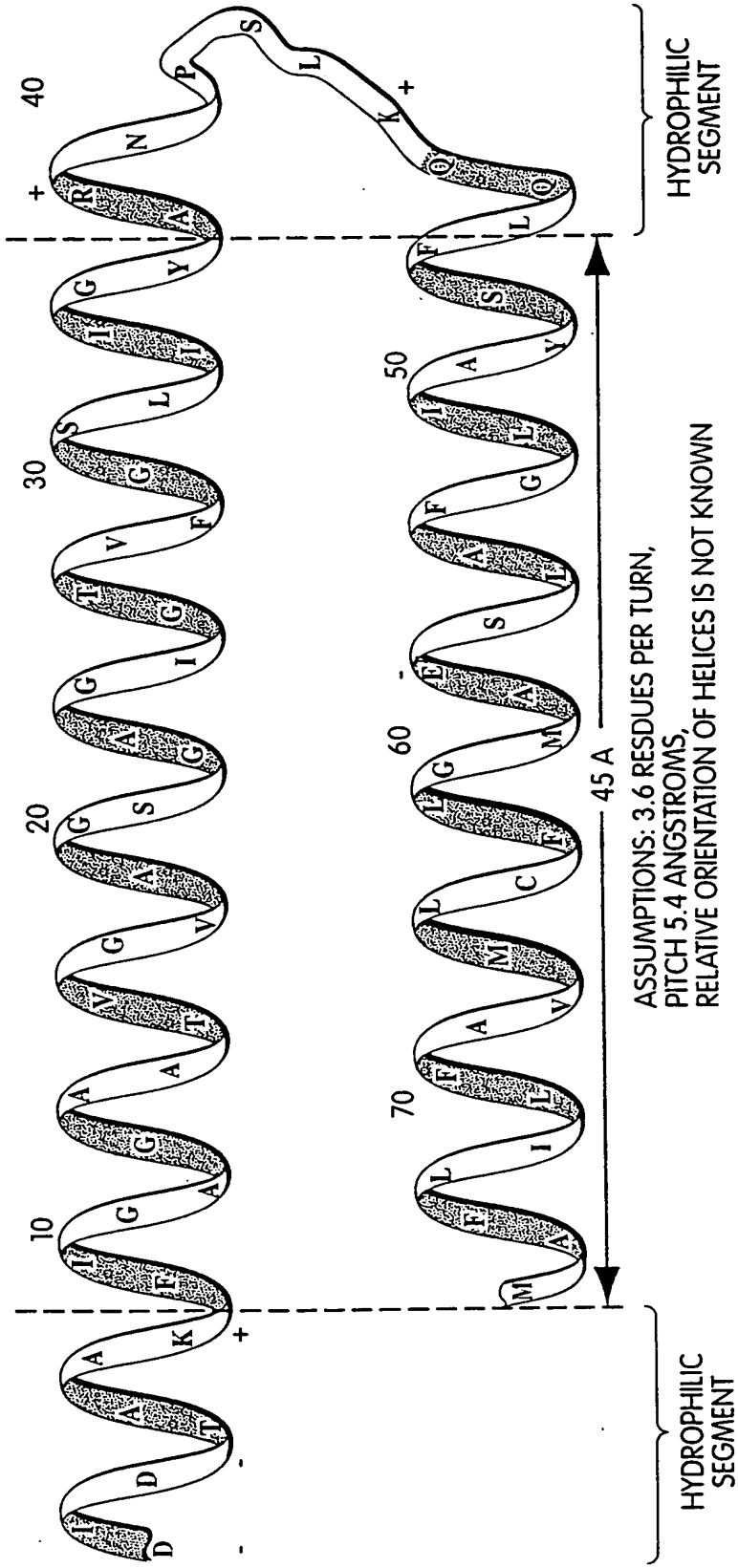

HYDROPHILIC SEGMENT
HYDROPHILIC SEGMENT
45 A
ASSUMPTIONS: 3.6 RESDUES PER TURN,
PITCH 5.4 ANGSTROMS,
RELATIVE ORIENTATION OF HELICES IS NOT KNOWN

Fig. 4B

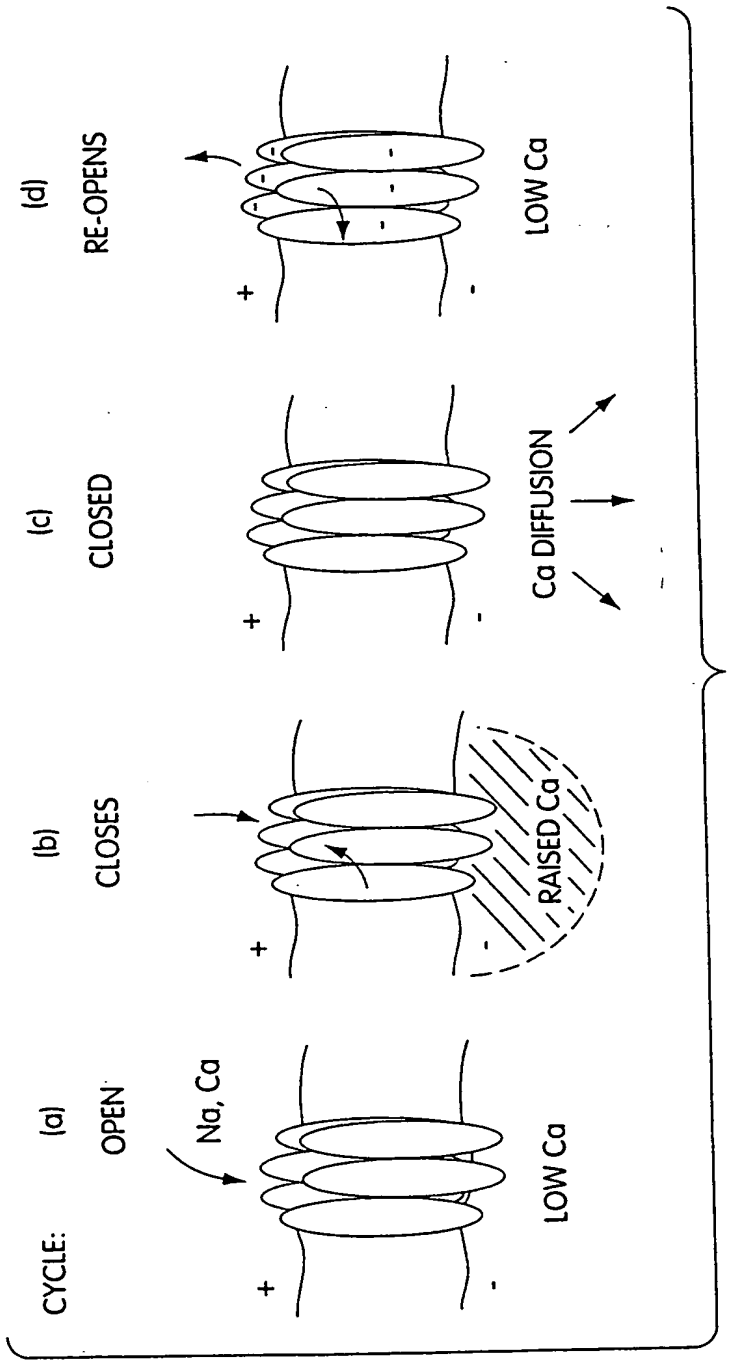

CYCLE:
(a)
OPEN
Na, Ca
LOW Ca
(b)
CLOSES
RAISED Ca
(c)
CLOSED
Ca DIFFUSION
(d)
RE-OPENS
LOW Ca

Fig. 5

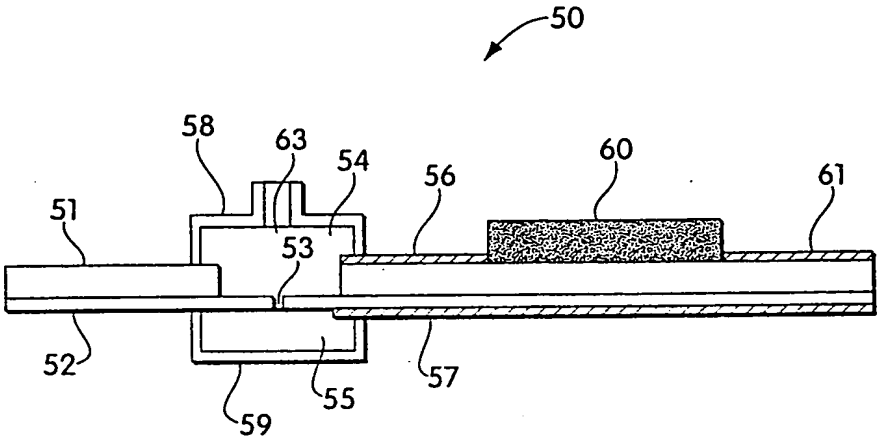


Fig. 6A

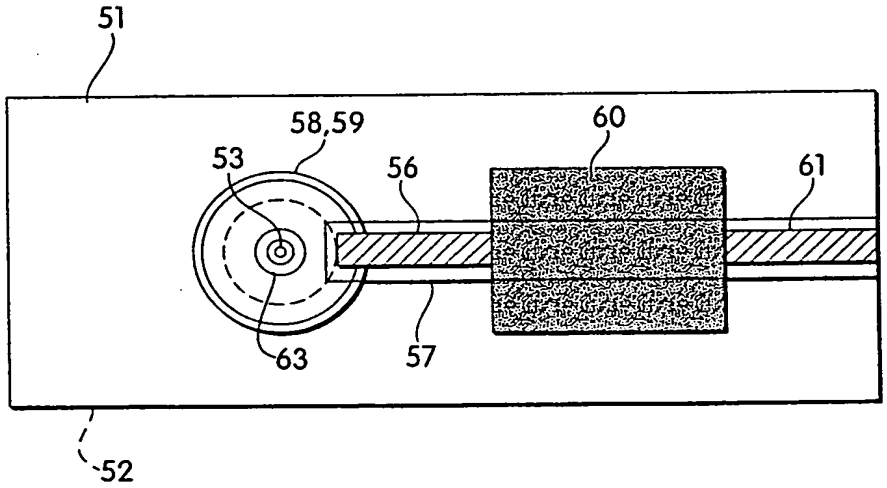


Fig. 6B

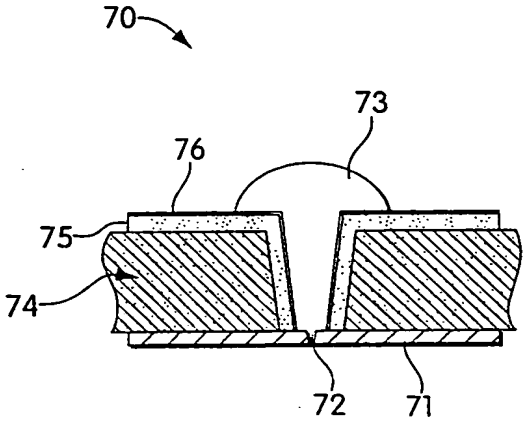


Fig. 7A

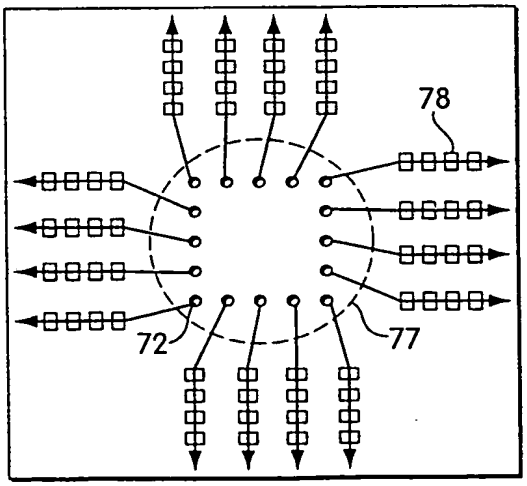


Fig. 7B

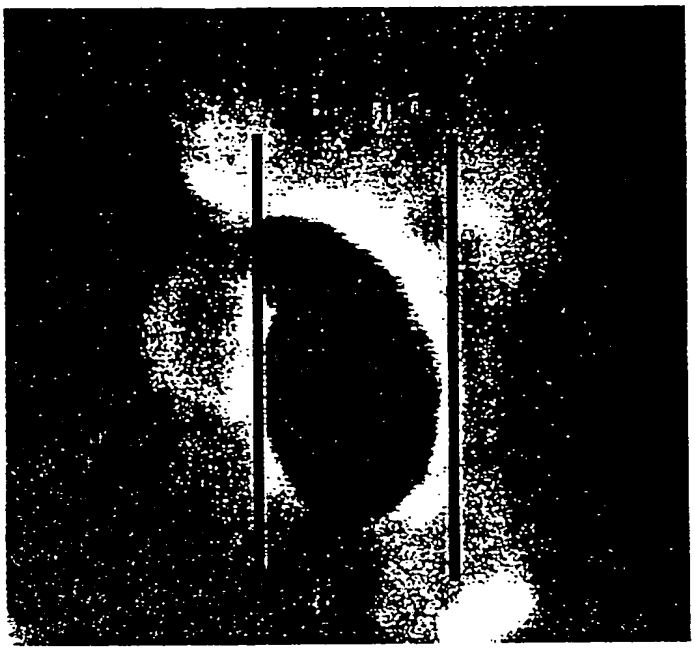


Fig. 8A

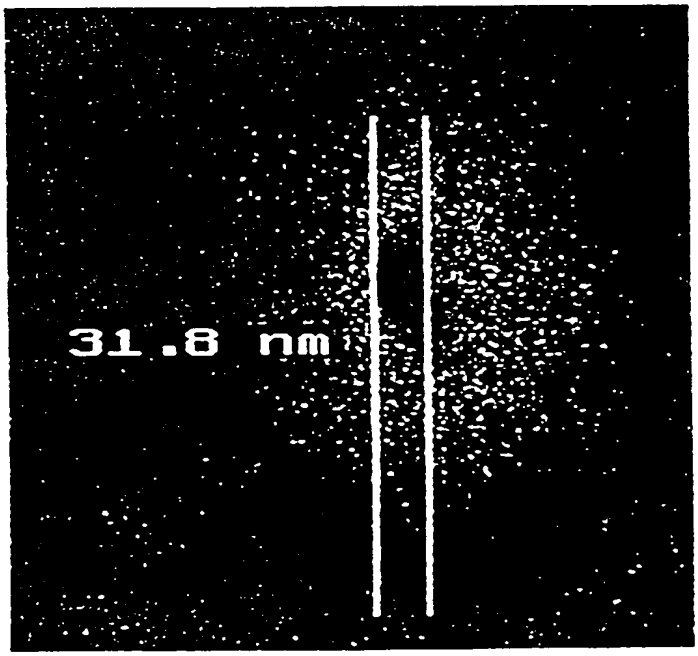


Fig. 8B

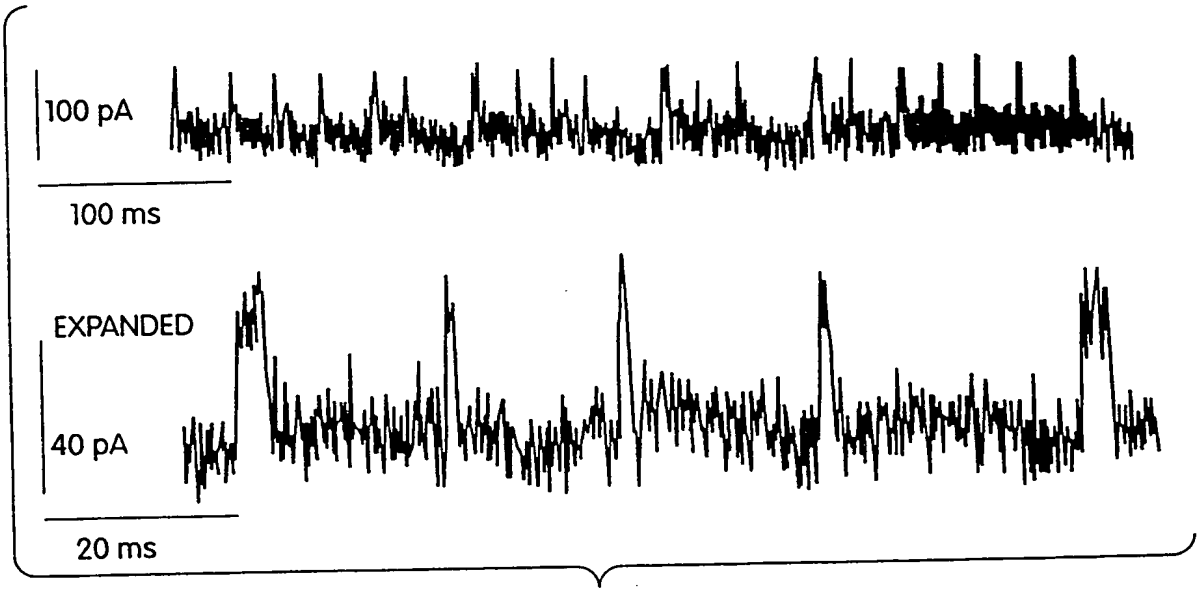


Fig. 9A

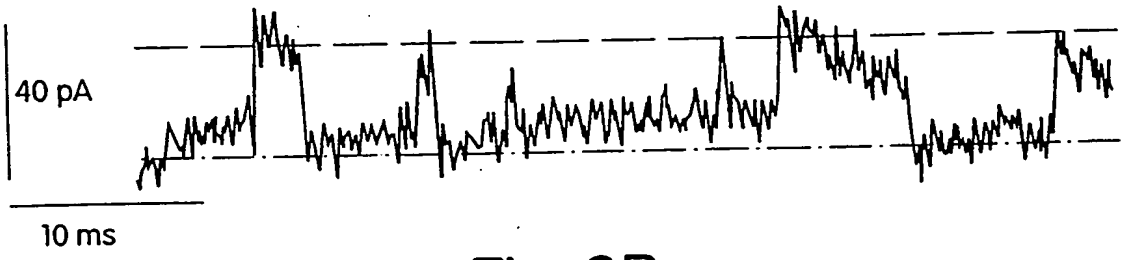


Fig. 9B

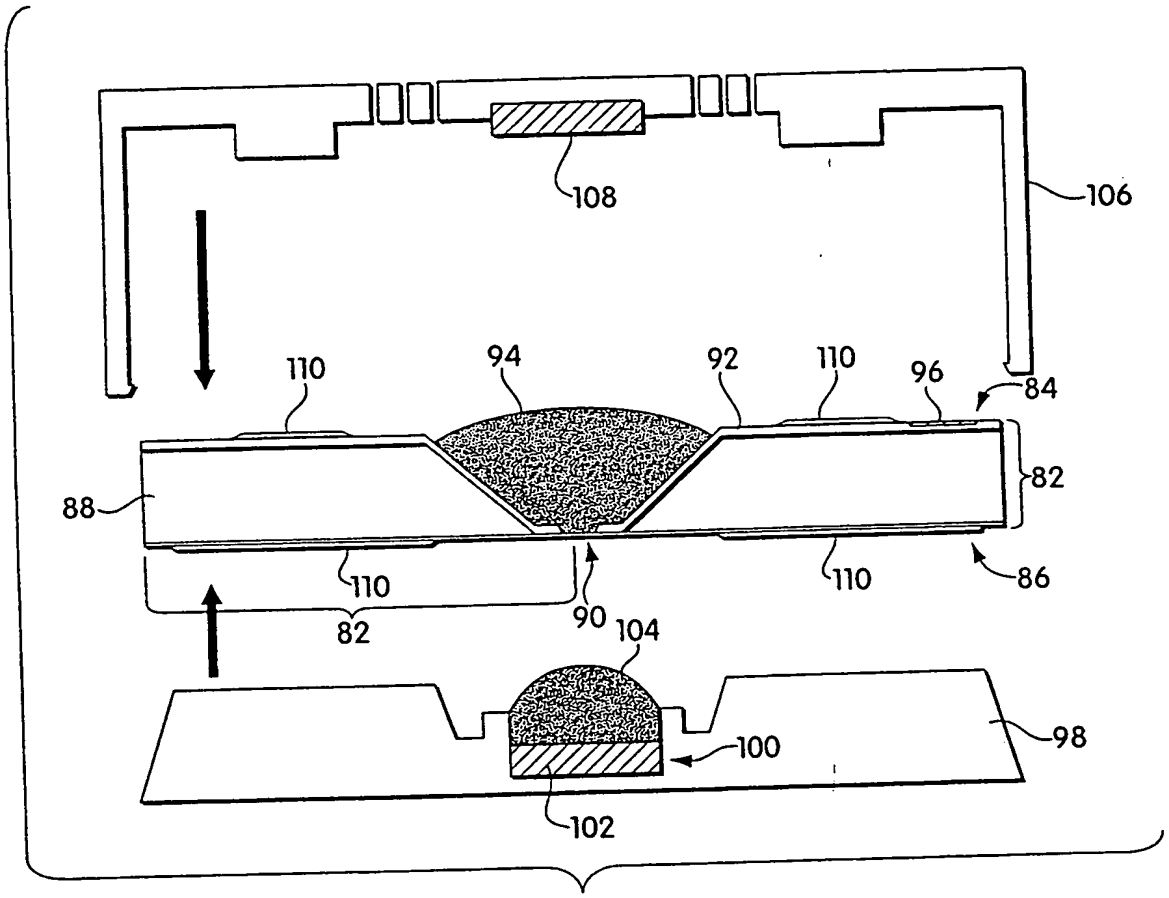


Fig. 10A

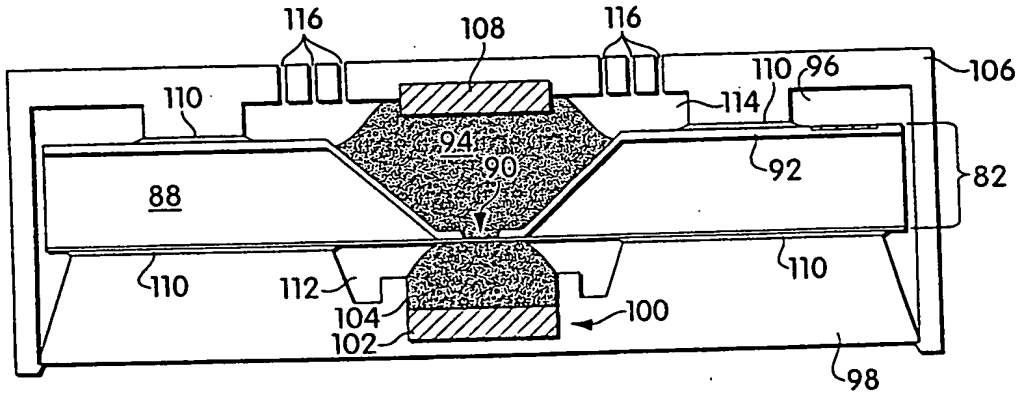


Fig. 10B

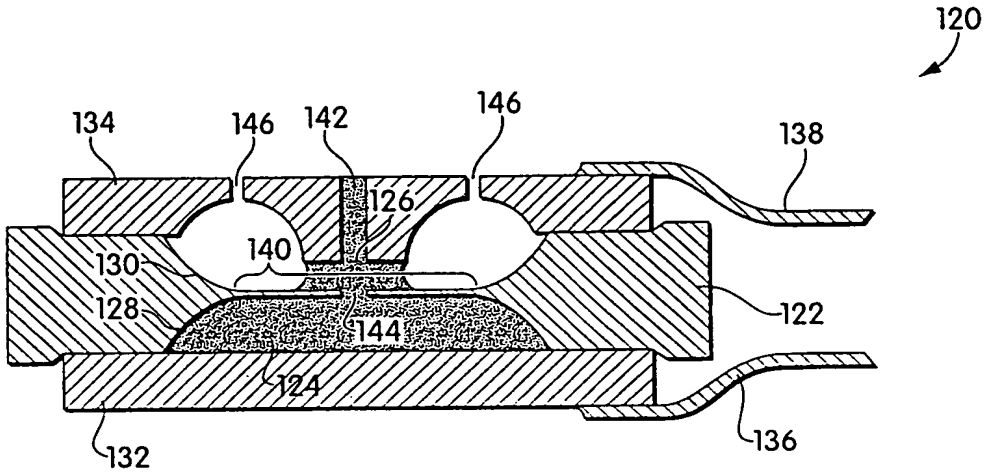


Fig. 11A

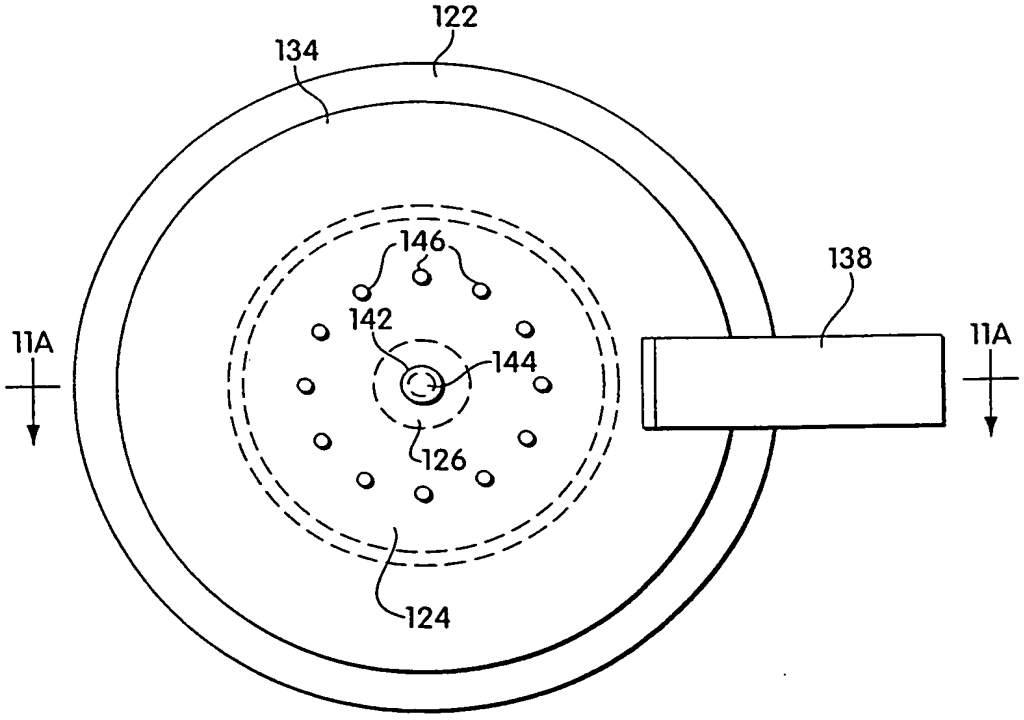


Fig. 11B

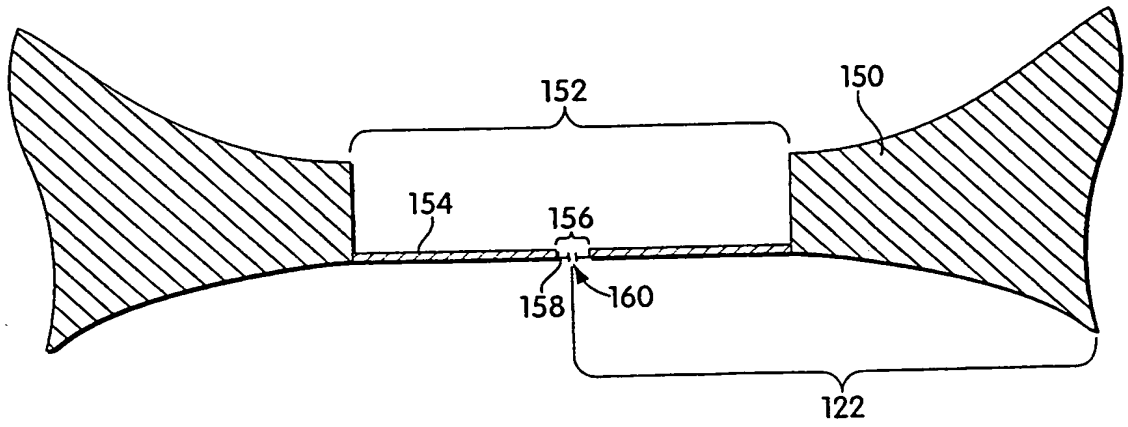


Fig. 12

INTERNATIONAL SEARCH REPORT

Inte\ onal Application No
PCT/US 99/24043

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N27/327 C12Q1/00 C12Q1/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MC GEOCH J.E.M, GUIDOTTI GUIDO: "A 0.1-700Hz current through a voltage-clamped pore: candidate protein for initiator of neural oscillations." BRAIN RESEARCH, vol. 766, no. 1-2, 1997, pages 188-194, XP000874820 cited in the application the whole document ---	1,8-21, 24, 26-40, 45-55
X	WO 94 25862 A (UNIV WASHINGTON) 10 November 1994 (1994-11-10) page 5, line 8-21 page 8, line 33 -page 9, line 18 figures --- -/--	1,2, 8-10, 24-28, 34,35, 46-52,54

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2000	11/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muñoz, M

3

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/24043

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			EP	0213825 A	11-03-1987
			JP	62098245 A	07-05-1987

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 15701 A (PASTERNAK CHARLES ALEXANDER ;BASHFORD CHARLES LINDSAY (GB); EDMOND) 21 July 1994 (1994-07-21) #22 figures 3,5,7 table 1 page 1, line 2 -page 2, line 15	1-5,17, 18,22, 24-28, 48-52
X	US 5 234 566 A (KING LIONEL G ET AL) 10 August 1993 (1993-08-10) #6 column 1, line 6-15 examples 1-9,13,16 figures column 5, line 43-54	1,8-10, 15,17, 22-24, 26-29, 34-40, 45-54
X	US 5 164 319 A (HAFEMAN DEAN G ET AL) 17 November 1992 (1992-11-17) #23 column 11, line 45 -column 14, line 23 column 15, line 14 -column 16, line 6	1,8-10, 22,23, 34,35, 46-54

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